Journal of Cereal Science 87 (2019) 194–201

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Impact of elevated CO_2 and drought on yield and quality traits of a historical (Blanqueta) and a modern (Sula) durum wheat

Gorka Erice[a], Álvaro Sanz-Sáez[b,1], Jon González-Torralba[c], Ana María Méndez-Espinoza[d], Inés Urretavizcaya[c], María Teresa Nieto[e], María Dolors Serret[f], José Luis Araus[f], Juan J. Irigoyen[g], Iker Aranjuelo[c,*]

[a] Departament of Soil Microbiology and Symbiotic Systems, Estación Experimental Del Zaidín (CSIC), Profesor Albareda No 1, 18008, Granada, Spain
[b] Division of Plant Sciences University of Missouri, 1-66 Ag Building, Columbia, MO, 65211, USA
[c] Instituto de Agrobiotecnología (IdAB), Universidad Pública de Navarra-CSIC- Gobierno de Navarra, Avenida Pamplona 123, E-31192, Mutilva Baja, Spain
[d] Plant Breeding and Phenomic Center, Faculty of Agricultural Sciences, Universidad de Talca, Talca, Chile
[e] Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Ctra. de La Coruña Km. 7.5, 28040, Madrid, Spain
[f] Integrative Crop Ecophysiology Group, Plant Physiology Section, Faculty of Biology, University of Barcelona and AGROTECNIO Center, Lleida. Av. Diagonal 645, 08028, Barcelona, Spain
[g] Department of Environmental Biology. Sciences and Pharmacy Faculties, University of Navarra, Irunlarrea 1, 31008, Pamplona, Spain

ARTICLE INFO

Keywords:
Climate change
Nutritional traits
Wheat
Yield

ABSTRACT

Wheat grain represents an important source of carbohydrates, proteins, lipids and minerals. Durum wheat is used mainly for the preparation of pasta, and in some Mediterranean areas is used for bread making. The atmospheric CO_2 concentration influences wheat growth, yield and quality. The present work focuses on kernel quality under conditions of elevated [CO_2] and subjected, or not, to water stress. The experiments were conducted with the durum wheat (*Triticum durum* Desf.) varieties cvv. Blanqueta, which is a historical Spanish landrace, and cvv. Sula, which is a modern variety. Sula demonstrated greater kernel weight (KW), insoluble protein (IP) content and amylose content, and also featured better potential test weight (TW) under projected future elevated [CO_2] and drought conditions. Blanqueta exposed to drought conditions showed the highest ^{13}C isotopic composition ($\delta^{13}C$) values indicating that, as a consequence of their higher biomass, they were subjected to a more severe stress. Under control conditions of ambient [CO_2], the protein concentrations of both varieties were similar. This work provides data about the genetic diversity between a currently cultivated wheat cultivar derived from traditional breeding and another cultivated some decades ago.

1. Introduction

Wheat is one of most consumed cereals in the diet of humans and animals, and it has been identified as a target crop in the context of food security. Wheat grain and its products are under progressively increasing demand worldwide, providing 18% of the food calories for the world's population and 19% of human daily protein consumption (FAO, 2013). Moreover, wheat grain also represents an important source of carbohydrates, lipids and minerals. Therefore, the importance of wheat proteins in the human diet should not be underestimated.

In order to be industrially processed, wheat grain composition must meet certain quality requirements, which depend on the characteristics of each end product. In the case of durum wheat, the main destination is the production of pasta, although it is also used for bread making in some areas in the Mediterranean region (Sissons, 2008). Some quality parameters are related to the grain itself, like grain weight, test weight and vitreousness, which determine the semolina yield (Troccoli et al., 2000). In addition, for pasta manufacturing, the quality of the grain composition is essential. Proteins play the most important role. They are responsible for conferring properties of viscosity and elasticity in the dough, thus providing a suitable texture during processing (Wieser, 2007). However, it is not only a matter of protein quantity, but also protein composition. The insoluble fraction of wheat grain protein, composed of gliadins and glutenins, exerts the most influence on the strength and elasticity of dough (Sissons, 2008). Starch is the major component of wheat endosperm and is mainly formed by two

* Corresponding author. Instituto de Agrobiotecnología (IdAB), Universidad Pública de Navarra-Consejo Superior de Investigaciones Científicas-Gobierno de Navarra, Avda. Pamplona 123, 31192, Mutilva, Navarra, Spain.
E-mail address: iker.aranjuelo@csic.es (I. Aranjuelo).
[1] Current Position: Department of Crop, Soil and Environmental Sciences, Auburn University, 253 Funchess Hall, Auburn, AL, 36849, USA.

https://doi.org/10.1016/j.jcs.2019.03.012
Received 21 June 2018; Received in revised form 26 October 2018; Accepted 13 March 2019
Available online 16 March 2019
0733-5210/

polysaccharides: amylose and amylopectin; amylose, is essentially linear and contributes 20–30% of total starch; amylopectin is highly branched and accounts for the remaining 70–80% (Lafiandra et al., 2010).

The nutritional composition and quality of wheat grain is not stable. Indeed, it is dependent on both genetic variability and the environmental conditions where wheat is grown. Several studies have demonstrated the influence of precipitation, mineral nutrition and atmospheric CO_2 concentration on wheat growth, yield and quality (Aranjuelo et al., 2013). In this context, Global Climate Change represents a major concern, particularly in the Mediterranean area, where durum wheat is more commonly cultivated. Along with a rise in [CO_2], in southern Europe a reduction in precipitation has been forecasted with [CO_2] reaching between 700 and 1000 μmol mol^{-1} for 2100 (IPCC, 2013, 2014). These changes can endanger durum wheat production and quality, which represents a threat to food security. Several studies have reported that an increment in [CO_2] (from 400 μmol mol^{-1} to elevated, 700 μmol mol^{-1}) increased wheat grain yields, but decreased the accumulation of storage proteins and the concentration of minerals in wheat grain (Aranjuelo et al., 2013; Nimesha et al., 2014). The reduction in grain protein content may have important economic and health implications (Högy and Fangmeier, 2008).

The response to elevated [CO_2] and water stress is highly dependent on genotypic variability. Moreover, it has been shown that old varieties may respond more strongly to [CO_2] enrichment than the modern ones because they possess more flexible traits to exploit that potential (Fois et al., 2011). Therefore, to confront these challenges, several breeding programs have started to recover old wheat varieties. The objective of this research was to study the effect of future climatic conditions, consisting of elevated [CO_2] and drought, on grain quality parameters and water use efficiency estimated by the δ^{13}C content of two varieties of durum wheat. One of these varieties, Blanqueta, is a historical Spanish landrace adapted to Mediterranean climates, whereas the other, Sula, is a modern variety that has been obtained by modern breeding selection techniques under similar climatic conditions.

2. Materials and methods

2.1. Wheat varieties and experimental design

The experiment was conducted with two durum wheat (*Triticum durum* Desf.) varieties (cvv. Sula and Blanqueta) cultivated in the Mediterranean region. Blanqueta is a historical Spanish landrace partially waxy for the WX-A1 locus (Nieto-Taladriz et al., 2000) while Sula is a modern (semidwarf) non-waxy Spanish variety released in 1994 with an origin from CIMMYT germoplasm (Chairi et al., 2018). The cultivars were kindly provided by the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA, Spain). Wheat seedlings vernalized for 1 month at 4 °C were transplanted into 13-L pots (four plants per pot) containing a mixture of inert 2 : 2: 1 (v/v/v) vermiculite: perlite: peat. The plants were grown at the Universidad de Navarra campus (42_80°N, 1_66°W; Pamplona, Spain) in greenhouses with [CO_2] control. Half of the plants (four pots of each cultivar per greenhouse) were divided between two greenhouses where no CO_2 was added and [CO2] was maintained at ambient conditions (≈400 μmol mol^{-1}). The other half of the plants (four pots of each cultivar per greenhouse) were transferred to two other greenhouses where the [CO_2] was increased to ≈700 μmol mol^{-1} by injecting pure CO_2 at the two inlet fans during daylight hours. Pots were rotated weekly to avoid edge effects. In order to avoid differences derived from chamber effects, the plants were moved from one greenhouse to the other one every month. Plants were watered with a complete Hoagland solution twice a week and with water once a week to avoid excessive salt accumulation. The plants were fully watered until anthesis. At the moment when at least 50% of the spikes per pot had reached anthesis (i.e. protrusion of 50% of anthers or spikes for each pot), the plants were randomly assigned to two water treatments. Within each greenhouse, half of the plants were labeled as well-watered plants (irrigated until pot capacity) and the other half as water-stressed plants. The maximum soil volumetric water content, corresponding to the well-irrigated treatments, was ~0.44 cm^3 cm^{-3}. The applied drought level corresponded to a 50% soil volumetric water content of well-watered plants (~0.22 cm^3 cm^{-3}). Water treatments were maintained until the end of the experiment when the plants reached maturity. Terminal drought was selected because it is characteristic of regions with Mediterranean-type climates where rainfall decreases, and evaporation and temperature increase in spring, when wheat enters its reproductive stage (Fitzpatrick and Nix, 1970).

2.2. Agronomic characterization

The number of days from sowing to anthesis was recorded for each variety and treatment. Anthesis was considered to take place when 50% of ears showed extruded anthers along the length of the head. At maturity, grain was harvested and kernel weight (KW) was determined. Test weight was calculated by weighing all grain produced and extrapolating to the pot's area.

2.3. C and N content and isotopic composition (δ^{13}C and δ^{15}N)

Around 7 days after anthesis (7 May), five representative flag leaves and ears were collected per pot, oven dried at 60 °C for 48 h, weighed and finely ground (1 mm) for carbon and nitrogen isotope analysis. Ground samples were weighed into tin capsules, sealed, and then sent for EA-IRMS analysis at the Scientific Facilities of the University of Barcelona. The ^{13}C/^{12}C ratios of plant material were expressed in δ notation:

$$\delta^{13}C = (R_{sample}/R_{standard})\text{-}1$$

The reference materials used in the δ^{13}C technique calibration were samples with known δ^{13}C (IAEA CH7 polyethylene foil, IAEA CH6 sucrose, and USGS 40 l-glutamic acid) calibrated against Vienna Pee Dee Belemnite calcium carbonate (VPDB) with an analytical precision (SD) of 0.10‰. The ^{15}N/^{14}N ratios were expressed in δ notation with respect to atmospheric N_2, with an analytical precision of 0.2‰: $\delta^{15}N = (R_{sample}/R_{standard})\text{-}1$.

The δ^{15}N accuracy was monitored using international secondary standards of known ^{15}N/^{14}N ratios (IAEA-N_1 and IAEA-N_2 ammonium sulfate and IAEA-NO_3 potassium nitrate, IAEA, Austria).

2.4. Kernel nutritional quality determinations

Kernel vitreousness (%) was determined using a Pohl farinator. a device that allows 50 wheat kernels to be held firmly while a blade is moved through to cut them transversely. The percentage of vitreous kernels is determined by examining the cross-section of the kernels. Vitreous grains appear dark and translucent, while opaque and non-vitreous grains appear yellow and starchy. The percentage vitreousness represents the average of the 50 kernels multiplied by 2.

Amylose content of wheat endosperm starch was estimated by the iodometric method (AACC 61–03.01) (AACC, 1983). Total nitrogen content was determined on ground dry grain material using an elemental analyzer (EA1108, Carbo Erba Instrumentazione, Corneredo, Italy) and multiplied by 5.7 to obtain protein content (%, db).

Insoluble grain protein content (%) was determined according to Suchy et al. (2007). On the other hand, gluten strength was estimated by measuring sedimentation volume, using the SDS-sedimentation test, determined as described by Axford et al. (1979).

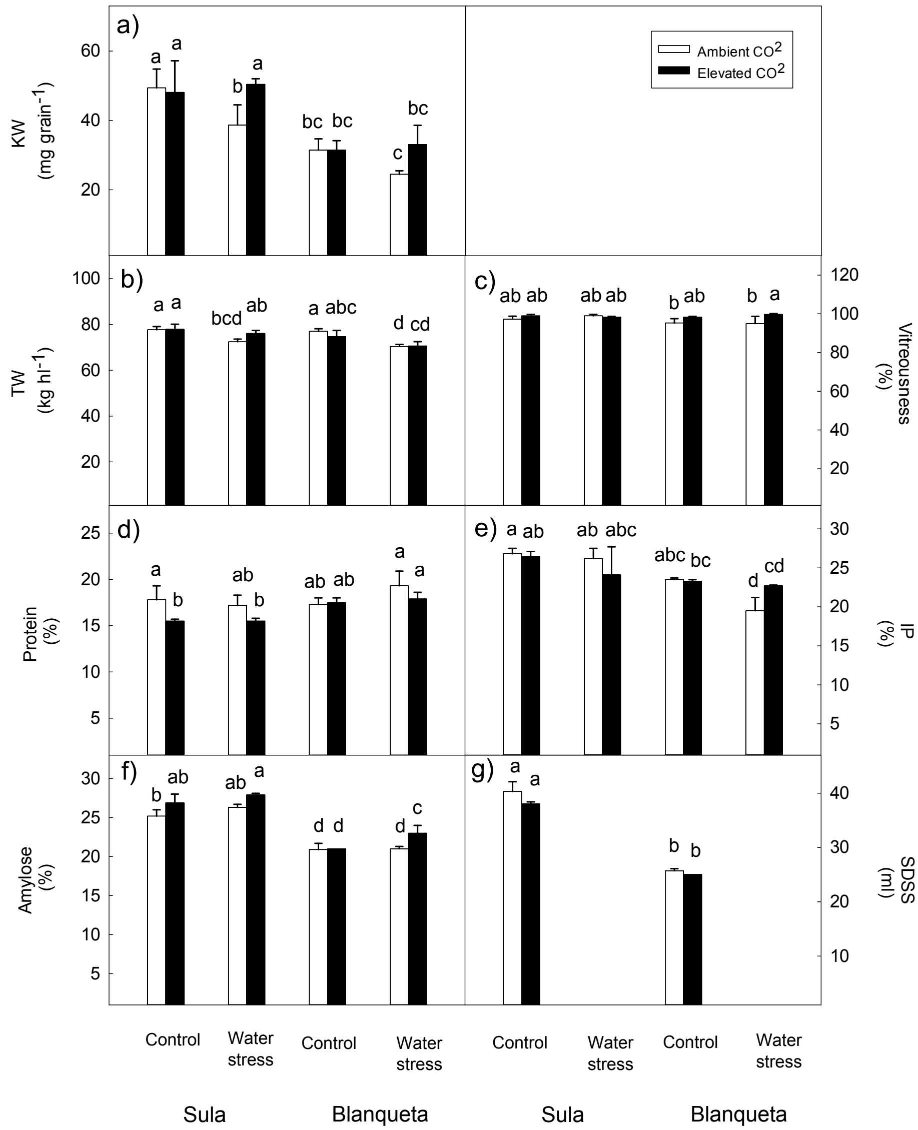

Fig. 1. Effect of elevated CO_2 concentration (700 µmol mol^{-1} vs 400 µmol mol^{-1}) and water availability (fully watered control vs water-stressed) on kernel weight (KW) (a), test weight (TW) (b), vitreousness (c), protein content (d), insoluble protein content (IP) (e), amylose content (f) and SDS sedimentation test (SDSS) (g) in Sula and Blanqueta wheat (*Triticum durum*) varieties. Each value represents the mean ± s.e. of three replications. Means within each subplot that differed significantly ($p < 0.05$) were followed by a different letter according to the LSD test parameters.

2.5. Statistical analyses

All the data were analyzed using the SPSS v. 19.0 statistical package (SPSS Inc., Chicago, IL, USA). The effect of different genotypes, [CO_2] and water availability on the agronomical and physiological parameters was tested with analysis of variance (ANOVA), performed using the general linear model (GLM) procedure. Different treatments were compared using the LSD post hoc test with $P < 0.05$ as the significance cut-off.

3. Results

3.1. Kernel quality, composition, and flour quality parameters

Mean kernel weight (KW) differed between the two varieties, with Sula displaying a higher value than Blanqueta (Fig. 1a; Table 1). This parameter clearly reflected the interaction between [CO_2] and the water regime: under ambient [CO_2], KW decreased by 22% in plants subjected to drought in both varieties; in contrast, no difference was observed between water regimes when plants were grown under elevated [CO_2] (Table 1). The exposure to elevated [CO_2] did not lead to an increase in KW in either Sula or Blanqueta when plants were grown in well-watered conditions. However, when plants subjected to

Table 1
Effect of CO_2 ([CO_2]), water regime (WR) and variety (V) on kernel weight (KW), test weight (TW), thousand kernel weight (TKW), vitreousness (Vitro), whole meal protein content (Prot), insoluble protein content (IP), amylose content (Amylose) and sedimentation volume (SDSS). The explanation for the symbols used in the ANOVA are as follows: ns, not significant differences; *, significant difference at 5%; **, significant difference at 1%; ***, significant difference at 0.1%.

Treatment	KW	TW	TKW	Vitro	Prot	IP	Amylose	SDSS
V	***	ns	***	ns	ns	**	***	***
[CO_2]	*	ns	*	*	*	ns	**	ns
R	ns	***	***	ns	ns	*	*	ns
Vx[CO_2]	ns	ns	***	ns	ns	*	***	ns
VxWR	ns	ns	***	ns	ns	ns	***	ns
[CO_2]xWR	*	ns	***	ns	ns	ns	ns	ns
Vx[CO_2]xWR	ns	*	**	ns	**	*	***	ns

drought were compared, elevated [CO_2] enhanced KW by 35% in Blanqueta and by 30% in Sula (Fig. 1a).

Test weight (TW) was clearly modified by water stress, displaying a mean reduction of 6% (Fig. 1b). However, the effect of drought differed depending on the [CO_2] treatment and variety (Table 1). Whereas Blanqueta exposed to drought conditions experienced a strong decrease in TW under both ambient and elevated [CO_2], in the case of Sula this trend was only observed under ambient [CO_2]. Contrary to this, there were no changes in TW under elevated [CO_2]. Thus, when Sula plants subjected to drought conditions were compared, there was a slight tendency for TW to increase under elevated [CO_2] (Fig. 1b).

Overall the mean vitreousness was 97.7%, with both varieties reaching very similar values: 98.4% for Sula and 97.1% for Blanqueta (Fig. 1c; Table 1). Elevated [CO_2] resulted in a significant 2% enhancement in the vitreousness, with this parameter not being affected by water stress or variety (Table 1). There were no significant differences in grain total soluble protein content between Blanqueta (18,0%) and Sula (16,5%) (Fig. 1d). While no effect of drought conditions was observed, elevated [CO_2] decreased grain protein content by 7% compared to ambient [CO_2] (Table 1). However, this effect varied depending on the variety and water regime. Protein content in Sula was reduced by 11% when exposed to elevated [CO_2], irrespective of the water regime. Blanqueta showed a greater stability, although at ambient [CO_2] a non-significant 12% increase was observed under drought in comparison to well-watered plants. As a result, Sula showed a lower protein content than Blanqueta when grown at elevated [CO_2] (Table 1). On the other hand, when plants were grown in drought conditions, both Blanqueta and Sula showed a non-significant tendency to decrease protein content under elevated [CO_2] (7% and 10%, respectively) (Fig. 1D).

The overall percentage of insoluble protein was higher in Sula (26%) compared to Blanqueta (22%) (Fig. 1e; Table 1). Plants subjected to drought conditions experienced an 8% decrease compared to well-watered plants. Although elevated [CO_2] did not alter the percentage of insoluble protein (IP), this parameter was affected by the interaction of [CO_2] with the variety and water stress (Table 1). When plants were grown in well-watered conditions, both varieties experienced a similar non-significant 1% decline in IP under elevated [CO_2] (Fig. 1e). In contrast, when plants were subjected to drought, Sula showed an 8% decrease in IP, whereas it increased in Blanqueta by 8% (Fig. 1e), although in both cases these changes were non-significant.

The amylose percentage was significantly higher in Sula compared to Blanqueta in all circumstances (26.6% vs. 21.5%) (Fig. 1f; Table 1). The exposure to drought conditions or elevated [CO_2] increased the amylose content by 4% and 7%, respectively, in comparison to well-watered conditions and ambient [CO_2]. Furthermore, plants subjected to elevated [CO_2] and drought demonstrated significant increases in their kernel amylose percentages (11% in Sula and 10% in Blanqueta) compared to ambient [CO_2] and normal irrigation conditions.

Sula displayed a higher sedimentation volume than Blanqueta (39.2% vs. 25.4%) (Table 1) and this quality trait was not significantly altered by [CO_2], although a slight tendency to decrease with elevated [CO_2] was observed for both varieties, especially in the case of Sula (Fig. 1g; Table 1).

3.2. Nitrogen and carbon content, and isotopic composition in different plant organs

The grain N concentration was significantly higher in the historical cultivar, Blanqueta (3.2%), than in Sula (2.9%) (Fig. 2; Table 2). In general, the nitrogen percentage was lower in grains of plants grown at elevated [CO_2] (Fig. 2; Table 2). However, the grain N concentration was not affected by the drought treatment. The ^{15}N isotope composition in grains ($\delta^{15}N$) was higher in Blanqueta compared to Sula, and was decreased by drought in both genotypes (Fig. 3; Table 2). In all organs, elevated [CO_2] decreased $\delta\ ^{13}C$, however, the effect of the water stress and genotype was variable depending on the organ (Table 2). In grain, $\delta\ ^{13}C$ decreased under elevated [CO_2] exposure and was enhanced by water stress treatment in both varieties (Fig. 3; Table 2). In addition, Blanqueta showed higher $\delta\ ^{13}C$ than Sula (Table 2).

As shown above for grain, the awn N was higher in Blanqueta than in Sula, although elevated [CO_2] led to N content depletion in both genotypes (Fig. 2; Table 2). Awn ^{15}N isotope composition was not affected by any treatment (Fig. 3; Table 2). Enhanced [CO_2] also increased the awn carbon content significantly (Fig. 2; Table 2). Elevated [CO_2] decreased the awn $\delta^{13}C$ in both genotypes (Fig. 3) but it remained higher overall in Blanqueta than in Sula. However, drought only increased the $\delta\ ^{13}C$ under elevated [CO_2] (Fig. 3; Table 2).

Elevated [CO_2] decreased the N content in all organs (Fig. 2; Table 2), however, only the $\delta^{15}N$ decreased in grain (Fig. 3). In addition, contrasting results were obtained for $\delta^{15}N$ regarding the water stress response for the different cultivars (Table 2). Drought led to enhanced $\delta^{15}N$ in Sula's flag leaf (24%), whereas in Blanqueta, $\delta^{15}N$ decreased by 11% (Fig. 3; Table 2). This distinct behavior between the varieties under different water regimes also featured in flag leaf C where Sula exhibited a 15% decline in C content, while it was enhanced by 6% in Blanqueta (Fig. 3; Table 2). Water stress only increased the δ13C in the flag leaves of Blanqueta plants grown under ambient [CO_2] conditions." (Table 2).

4. Discussion

The forecasted increase in atmospheric CO_2 concentration is expected to have a major impact on crop production in the near future. The effect of elevated [CO_2] in wheat has been extensively studied regarding yield and quality (Fares et al., 2016; Panozzo et al., 2014). However, further knowledge, especially concerning the joint effect of elevated [CO_2] and drought during the grain filling period is still needed because low water availability is a common feature of Mediterranean-type climates, and this situation may be exacerbated by climate change. Earlier work comparing the response of the wheat varieties Sula and Blanqueta demonstrated that Sula, featuring a higher harvest index (HI), can respond better to elevated [CO_2] in terms of both grain and shoot biomass production (Aranjuelo et al., 2013). The present work focuses on kernel quality under conditions of elevated [CO_2] and subjected or not to water stress.

The study showed that neither Sula nor Blanqueta were able to increase their kernel weight under elevated [CO_2] conditions when there was no shortage of water supply. This result confirms that the yield increase in response to elevated [CO_2] previously observed in Sula (Aranjuelo et al., 2013) was a consequence of an increase in grain number but not grain weight. Panozzo et al. (2014), in tree wheat cultivars, also found an increase in grain yield, associated to thousand grain weigh (TGW) by an increase in grain size. Other authors have also reported no effect of increased [CO_2] on kernel weight (Högy and Fangmeier, 2008). However, both increases and decreases (Panozzo et al., 2014) in kernel weight under elevated [CO_2] have been described, suggesting the co-influence of other factors in this quality trait, as is the case for sowing date (Fernando et al., 2014). One of the other factors that can influence the response, is water availability; when plants were subjected to water stress, the increase in [CO_2] was able to compensate for the effect of stomatal closure, thus maintaining the rate of CO_2 assimilation as well as starch synthesis and deposition in the grain. This trend of kernel weight gain with increasing [CO_2] under drought but not in well-watered conditions is in agreement with the results obtained by Fernando et al. (2014).

Natural $\delta^{15}N$ and $\delta^{13}C$ in plant biomass in combination with N content have been used as physiological tracers of N absorption through the roots and C assimilation by the photosynthetic apparatus (Bort et al., 2014). Elevated [CO_2] usually decreased biomass N content in C_3 plants, and it is usually associated with a dilution effect, sink limitation, and reduced NO_3^- photo-assimilation under elevated [CO_2] (Aranjuelo

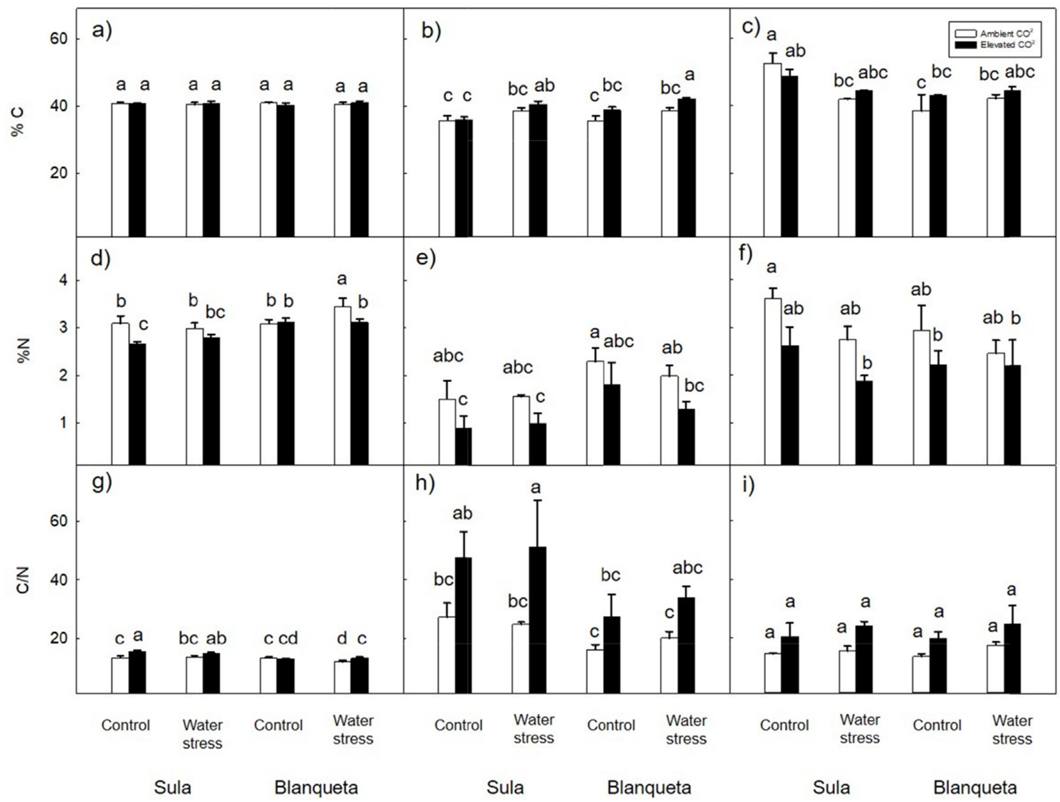

Fig. 2. Effect of elevated CO_2 concentration (700 μmol mol^{-1} vs 400 μmol mol^{-1}) and water availability (fully watered control vs water-stressed) on carbon percentage (%C) in grain (a), awn (b) and flag leaf (c); nitrogen percentage (%N) in grain (d), awn (e) and flag leaf (f); and C/N ratio (C/N) in grain (g), awn (h) and flag leaf (i) in Sula and Blanqueta wheat (*Triticum durum*) varieties. Each value represents the mean ± s.e. of three replications. Means within each subplot that differed significantly ($p < 0.05$) were followed by a different letter according to the LSD test parameters.

et al., 2013). In our study, only Sula grown under elevated [CO_2] showed significant decreases in grain N content under both water regimes. As in previous experiments, this variety showed higher sink capacity than Blanqueta under elevated [CO_2], and because the N source was the same for both varieties, the drop in N content in this variety can only be attributed to a dilution effect caused by greater biomass and grain weight accumulation (Aranjuelo et al., 2013). Some authors have suggested that shifts in $\delta^{15}N$ values could indicate metabolic change and/or low N availability because N absorption and mobilization is not able to discriminate against the ^{15}N isotope when N content is low, and thus $\delta^{15}N$ is increased (Bort et al., 2014). In this study, elevated [CO_2] tended to increase leaf and grain $\delta^{15}N$ in both varieties, but with slightly higher values in Sula, reinforcing the idea of N dilution effects in this variety.

The kernels and (at least for Blanqueta) the flag leaves and awns of plants under water stress exhibited slightly lower $\delta^{15}N$ values than the well-watered plants at atmospheric [CO_2], while the pattern was less clear at high [CO_2]. Decreases in $\delta^{15}N$ following water limitation (Bort et al., 2014) or under growing conditions causing water stress have been reported before in wheat. However, the effect of rising [CO_2] on $\delta^{15}N$ was less clear than the effect of the water regime. Rising [CO_2] increased and decreased the isotopic composition in the kernels and awns, respectively, whereas in the flag leaf the opposite trend was found. Depletion of the heavier N isotope in plants grown under high [CO_2] and water deficit conditions has been reported before in studies with alfalfa (Ariz et al., 2015) and sweet pepper (Serret et al., 2018). The decrease in $\delta^{15}N$ under elevated [CO_2] may reflect decreased stomatal conductance, but could also be related to a higher nitrogen demand in leaves, as suggested by the decrease in leaf N (Ariz et al., 2015) or shifts in nitrogen metabolism associated with decreases in photorespiration. The increase in $\delta^{15}N$ in response to higher [CO_2] in kernels may be associated with N accumulation in kernels being the result of translocation from the photosynthetic organs (leaves and spikes).

The evaluation of $\delta^{13}C$ is an indirect way to determine water use efficiency (WUE). In this study high CO_2 decreased the $\delta^{13}C$ in both cultivars, with a stronger response in the grain and awns than in the flag leaf. In terms of the part of the plant, it has been demonstrated that the carbon isotope composition in grains is lower than that of leaves. Water stress increased the $\delta^{13}C$ in the grain of Sula and in the awns of both cultivars, which agrees with the findings of Serret et al. (2018) in leaves of sweet pepper. Under normal CO_2, the water stress treatment also increased the $\delta^{13}C$ in the grain, and in flag leaves of Blanqueta compared to the control, which probably resulted from reductions in stomatal conductance that were associated with water preservation (Araus et al., 2002). In this regard, Blanqueta, the low yielding old cultivar, showed higher $\delta^{13}C$ than Sula, the high yielding newer cultivar, implying that Blanqueta was subjected to a more severe stress. Previous reports in grains and whole spikes of durum wheat under normal CO_2 conditions have reported an increase in the $\delta^{13}C$ in the grains and whole spikes as response to water stress, with modern varieties exhibiting lower (i.e. more negative) values than the landraces and old cultivars (Araus et al., 2013; Sanchez-Bragado et al., 2014; Vicente et al., 2018). The higher biomass of Blanqueta would imply higher transpiration rates with a consequent effect on soil water consumption.

Test weight is a valued quality trait because it is related to milling yield and also reflects the soundness of the grain (Troccoli et al., 2000). In the present study, test weight (TW) was unaffected by elevated [CO_2] in both varieties, in agreement with Panozzo et al. (2014), but in general the TW decreased with drought, probably as a result of changes in kernel diameter (Fernando et al., 2014). However, no decrease in TW with drought was observed in the case of Sula grown under elevated [CO_2]. Therefore, the new cultivar Sula seems to be better adapted to

Table 2
Effect of CO_2 [CO_2], water regime (WR) and variety (V) on N, $\delta^{15}N$, C and $\delta^{13}C$ of grains, awns and flag leaf. The explanation for the symbols used in the ANOVA are as follows: ns, not significant differenc]s; *, significant difference at 5%; **, significant difference at 1%; ***, significant difference at 0.1%.

Factor	Grain			
	N	$\delta^{15}N$	C	$\delta^{13}C$
V	***	ns	ns	**
[CO_2]	**	*	ns	***
WR	ns	*	ns	***
Vx[CO_2]	ns	ns	ns	ns
VxWR	ns	ns	ns	ns
[CO_2]xWS	ns	ns	ns	ns
Vx[CO_2]xWR	ns	ns	ns	ns
Factor	**Awn**			
	N	$\delta^{15}N$	C	$\delta^{13}C$
V	**	ns	ns	**
[CO_2]	**	ns	**	***
WR	ns	ns	***	***
Vx[CO_2]	ns	ns	ns	**
VxWR	ns	ns	ns	ns
[CO_2]xWR	ns	ns	ns	**
Vx[CO_2]xW	ns	ns	ns	ns
Factor	**Flag Leaf**			
	N	$\delta^{15}N$	C	$\delta^{13}C$
V	ns	ns	*	ns
[CO_2]	*	ns	ns	***
WS	ns	ns	ns	ns
Vx[CO_2]	ns	ns	ns	ns
VxWS	ns	*	*	ns
CO_2xWS	ns	ns	ns	ns
Vx[CO_2]xWS	ns	ns	ns	ns

future climatic conditions than Blanqueta.

The percentage of vitreous kernels is a key quality parameter for durum wheat because it is related to the semolina yield, which has high vitreousness values and indicates the quality of the wheat for pasta production (Sissons et al., 2000). In the present study, the recorded values were very high for the two varieties irrespective of the growing conditions. Nevertheless, a positive effect of elevated [CO_2] on vitreousness was detected, suggesting that pasta-making characteristics for durum wheat will be improved in a future with increased atmospheric [CO_2].

Wheat grains are not only a source of carbohydrates, but also of proteins, amino acids, minerals and lipids (Shewry, 2009). Concerning the kernel composition, both varieties displayed similar protein content when cultivated under ambient [CO_2] and full water availability. However, they responded differently to elevated [CO_2]. Protein content (%) decreased in Sula in both water regimes while it remained stable in Blanqueta. A reduction in grain nitrogen content under elevated [CO_2] has been previously observed (Erbs et al., 2010; Högy and Fangmeier, 2008). This reduction has been related to an inhibition of leaf assimilation of nitrate into organic compounds due to the reduction in photorespiration rates (Bloom et al., 2014). In comparison, the old cultivar Blanqueta displayed a more stable pattern, although a non-significant increase in the protein content was detected under ambient [CO_2] and drought, which could be related to the reduced kernel weight in that situation. Better responses of old varieties to elevated [CO_2] regarding protein content have been reported previously. Fares et al. (2016) observed a decrease in protein content in a wide range of varieties but, interestingly, one of the oldest varieties displayed the lowest reduction. Fois et al. (2011) found that under different sowing times there was also a clear trend of decreasing average protein contents from 'old' to 'modern' cultivars. The present results indicate that the historical variety, Blanqueta, may be better adapted to maintain grain protein content in future climate conditions. In our case, following exposure to elevated [CO_2], Sula had the lowest protein content of around 15%, which was above the lowest recommended limit value, but this finding may question the ability of modern varieties like Sula to supply grain that can fulfill the quality requirements of the industry and consumers in the future. Increased nitrogen fertilization might partly counterbalance this reduction in protein content, but cannot completely mitigate it (Erbs et al., 2010). In this sense, it is important to ensure that wheat will maintain its kernel protein content and hence nutritional and quality value under future climate conditions.

Although protein content is the main criterion regarding grain quality, protein composition must be also considered. Among the kernel proteins, insoluble protein (IP) constitutes the most relevant fraction in terms of protein quality. IP includes the monomeric gliadins and polymeric glutenins, which are constituents of gluten. They are responsible for conferring viscoelastic behavior (Shewry, 2009) and contributing to dough strength (Wieser, 2007). It is generally considered that IP is the main quality indicator of semolina for pasta making (Troccoli et al., 2000). Protein and gluten levels determine dough extensibility, a desirable feature during industrial processing of durum wheat flour, because gluten must retain the gelatinized starch granules during pasta cooking (Soh et al., 2006). IP was higher in Sula compared to Blanqueta, irrespective of the [CO_2] and water regime. Furthermore, IP in Sula was not modified by any treatment. In contrast, Blanqueta displayed a significant decrease in IP in plants grown under ambient [CO_2] and subjected to drought in comparison to the well-watered ones. Thus, in spite of the stability of Blanqueta in terms of protein content, its protein quality may deteriorate under drought conditions. Nevertheless, in plants grown in elevated [CO_2], no differences were observed between well-watered plants and those subjected to drought in either Sula or Blanqueta. Therefore, elevated [CO_2] may not have affected this quality parameter in the studied durum wheat varieties. SDS sedimentation test (SDSS) volume is used to predict gluten quality. In this case, Sula displayed higher volumes than Blanqueta, thus implying a higher gluten quality. No change was observed with respect to [CO_2] conditions, despite the decline in protein content in Sula, which can be related to the invariability of IP under elevated atmospheric [CO_2]. The lack of response of the SDSS test to [CO_2] variation is in accordance with previous findings (Fares et al., 2016).

Starch also plays an important role in the determination of the quality traits of durum wheat for pasta making, with a high starch concentration being considered as a positive trait (Högy and Fangmeier, 2008). Nevertheless, starch properties such as the amylose/amylopectin ratio are mostly neglected in CO_2 experiments (Högy and Fangmeier, 2008). Comparisons between Sula and Blanqueta highlighted that the modern cultivar had a higher amylose percentage in kernel starch under all growing conditions. Blanqueta is a partial waxy variety, null for the *Wx-B1* locus, thus featuring significantly lower overall amylose content than Sula. Elevated [CO_2] and drought showed a slight tendency to enhance the amylose percentage, the most relevant increase being obtained by the combination of both conditions. Elevated [CO_2] has been related to increased amylograph peak viscosity values of wheat starch (Panozzo et al., 2014), which can be promoted by a higher amylose content (Soh et al., 2006). This increase in amylose content could lead to reduced dough elasticity and extensibility (Van Hung et al., 2005; Soh et al., 2006), but has a positive impact on the pasta industry as it reduces starch loss during cooking and increases pasta firmness.

5. Conclusion

In conclusion, the two wheat cultivars in this study showed opposite behavior regarding kernel quality and water use efficiency when subjected to projected future atmospheric [CO_2] levels. Sula demonstrated greater kernel weigh, insoluble protein, amylose content, and SDS sedimentation, and lower water use efficiency. Moreover, Sula also featured better potential test weight under future climate conditions of

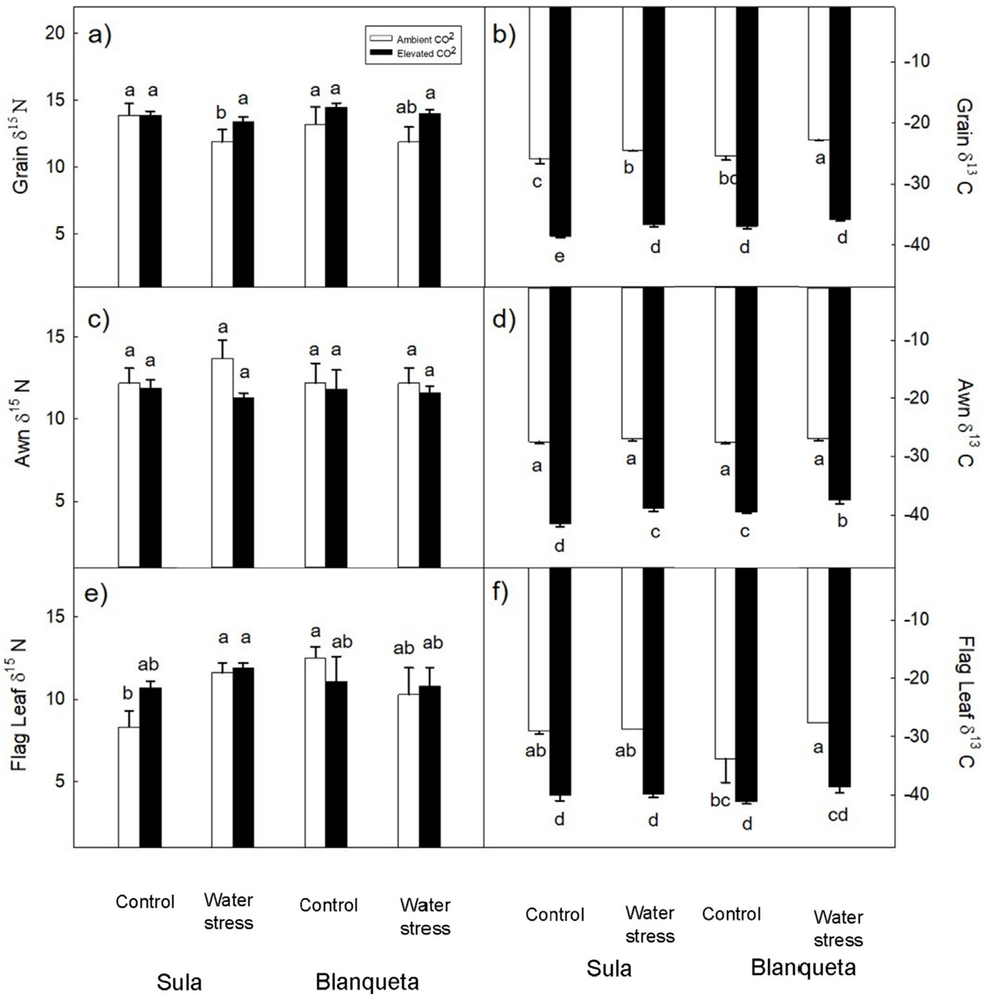

Fig. 3. Effect of elevated CO_2 concentration (700 μmol mol^{-1} vs 400 μmol mol^{-1}) and water availability (fully watered control vs water-stressed) on Grain δ^{15}N (a), Grain δ^{13}C (b), Awn δ^{15}N (c), Awn δ^{13}C (d), Flag Leaf δ^{15}N (e) and Flag Leaf δ^{13}C (f) in Sula and Blanqueta wheat (*Triticum durum*) varieties. Each value represents the mean ± s.e. of three replications. Means within each subplot that differed significantly ($p < 0.05$) were followed by a different letter according to the LSD test parameters.

elevated CO_2 and drought. Under control conditions of ambient [CO_2], the protein concentration of both varieties was similar. However, Blanqueta exhibited a lower decrease in kernel protein content and insoluble protein under the combined simulated future CO_2 and drought conditions. This highlighted that Blanqueta, the traditional wheat landrace, is less susceptible to suffering from loss of kernel protein nutritional value. On the other hand, the highest δ^{13}C values detected in Blanqueta exposed to drought conditions showed that these plants were subjected to a higher water stress. Such an effect was associated with a higher biomass and consequently higher transpiration rates. In this work, it can be appreciated that although Sula shows better agronomic response to elevated [CO_2]; Blanqueta showed a lower decreased of quality traits (protein and insoluble protein), under drought and elevated [CO_2]. Although old cultivars might not have the best agronomic traits, they might add some interesting characteristics from the point of view of grain quality or stress tolerance traits, when incorporated in breeding programs. Obtained results remark that, in order to better understand the influence of different environmental conditions in grain yield and quality traits, further research is needed using a bigger set of genotypes under different environmental conditions.

Acknowledgements

The authors would like to thank Messrs. Amadeo Urdiain and Hector Santesteban for technical support with managing the greenhouse. This work was supported by the Spanish Ministry of Science and Innovation (AGL2016-79868-R). JLA, acknowledges the Support from ICREA Academia, Government of Catalonia, Spain.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcs.2019.03.012.

References

AACC, 1983. Approved Methods of the AACC, ninth ed. American Association of Cereal Chemists, St Paul, MN Method 54-30A.
Aranjuelo, I., Sanz-Sáez, A., Jauregui, I., Irigoyen, J.J., Araus, J.L., Sánchez-Díaz, M., Erice, G., 2013. Harvest index, a parameter conditioning responsiveness of wheat plants to elevated CO_2. J. Exp. Bot. 64, 1879–1892.
Araus, J., Slafer, G., Reynolds, M., Royo, C., 2002. Plant breeding and drought in C-3 cereals: what should we breed for? Ann. Bot. 89, 925–940.
Araus, J.L., Cabrera-Bosquet, L., Serret, M.D., Bort, J., Nieto-Taladriz, M.T., 2013. Comparative performance of δ^{13}C, δ^{18}O and δ^{15}N for phenotyping durum wheat adaptation to a dryland environment. Funct. Plant Biol. 40, 595–608.
Ariz, I., Cruz, C., Neves, T., Irigoyen, J.J., García, C., Nogués, S., Aranjuelo, I., 2015. Leaf δ^{15}N as a physiological indicator of the responsiveness of N_2-fixing alfalfa plants to elevated [CO_2], temperature and low water availability. Front. Plant Sci. 6, 574.
Axford, D., Mcdermott, E., Redman, D., 1979. Sodium dodecyl-sulfate test of bread-making quality - comparison with pelshenke and zeleny tests. Cereal Chem. 56, 582–584.

Bloom, A.J., Burger, M., Kimball, B.A., Pinter Jr., P.J., 2014. Nitrate assimilation is inhibited by elevated CO_2 in field-grown wheat. Nat. Clim. Change 4 (6), 477.

Bort, J., Belhaj, M., Latiri, K., Kehel, Z., Araus, J.L., 2014. Comparative performance of the stable isotope signatures of carbon, nitrogen and oxygen in assessing early vigour and grain yield in durum wheat. J. Agric. Sci. 152 (3), 408–426.

Chairi, F., Vergara-Diaz, O., Vatter, T., Aparicio, N., Nieto-Taladriz, M.T., Kefauver, S.C., Bort, J., Serret, M.D., Araus, J.L., 2018. Post-green revolution genetic advance in durum wheat: the case of Spain. Field Crop. Res. 228, 158–169.

Erbs, M., Manderscheid, R., Jansen, G., Seddig, S., Pacholski, A., Weigel, H.J., 2010. Effects of free-air CO_2 enrichment and nitrogen supply on grain quality parameters and elemental composition of wheat and barley grown in a crop rotation. Agric. Ecosyst. Environ. 136 (1–2), 59–68.

FAO, 2013. FAO Statistical Database (Rome: Food and Agriculture Organization of the United Nations). Online: http://faostat3.fao.org/home/index.html.

Fares, C., Menga, V., Badeck, F., Rizza, F., Miglietta, F., Zaldei, A., Codianni, P., Iannucci, A., Cattivelli, L., 2016. Increasing atmospheric CO_2 modifies durum wheat grain quality and pasta cooking quality. J. Cereal Sci. 69, 245–251.

Fernando, N., Panozzo, J., Tausz, M., Norton, R.M., Neumann, N., Fitzgerald, G.J., Seneweera, S., 2014. Elevated CO_2 alters grain quality of two bread wheat cultivars grown under different environmental conditions. Agric. Ecosyst. Environ. 185, 24–33.

Fitzpatrick, E.A., Nix, H.A., 1970. Climatic Factor in Australian Grassland Ecology. (Moore, RM Australian grasslands).

Fois, S., Schlichting, L., Marchylo, B., Dexter, J., Motzo, R., Giunta, F., 2011. Environmental conditions affect semolina quality in durum wheat (*Triticum turgidum* ssp *durum* L.) cultivars with different gluten strength and gluten protein composition. J. Sci. Food Agric. 91, 2664–2673.

Högy, P., Fangmeier, A., 2008. Effects of elevated atmospheric CO_2 on grain quality of wheat. J. Cereal Sci. 48 (3), 580–591.

IPCC, 2013. Climate Change 2013: the Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. (Cambridge, United Kingdom and New York, NY, USA).

IPCC, 2014. Climate change 2014: synthesis report. In: Core Writing Team, Pachauri, R.K., Meyer, L.A. (Eds.), Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, pp. 151.

Lafiandra, D., Sestili, F., D'Ovidio, R., Janni, M., Botticell, E., Ferrazzano, G., Silvestri, M., Ranieri, R., DeAmbrogio, E., 2010. Approaches for modification of starch composition in durum wheat. Cereal Chem. 87 (1), 28–34.

Nieto-Taladriz, M.T., Rodríguez-Quuano, M., Carrillo, J.M., 2000. Polymorphism of waxy proteins in Spanish durum wheats. Plant Breed. 119 (3), 277–279.

Nimesha, F., Panozzo, J., Tausz, M., Norton, R.M., Fitzgerald, G.F., Myers, S., Nicolas, M.E., Seneweera, S., 2014. Intra-specific variation of wheat grain quality in response to elevated [CO2] at two sowing times under rain-fed and irrigation treatments. Agric. Ecosyst. Environ. 59 (2), 137–144.

Panozzo, J.F., Walker, C.K., Partington, D.L., Neumann, N.C., Tausz, M., Seneweera, S., Fitzgerald, G.J., 2014. Elevated carbon dioxide changes grain protein concentration and composition and compromises baking quality. A FACE study. J. Cereal Sci. 60 (3), 461–470.

Sanchez-Bragado, R., Elazab, A., Zhou, B., Serret, M.D., Bort, J., Nieto-Taladriz, M.T., Araus, J.L., 2014. Contribution of the ear and the flag leaf to grain filling in durum wheat inferred from the carbon isotope signature: genotypic and growing conditions effects. J. Integr. Plant Biol. 56, 444–454.

Serret Molins, M.D., Yousfi, S., Vicente García, R., Piñero, M.C., Otálora-Alcón, G., del Amor, F.M., Araus Ortega, J.L., 2018. Interactive effects of CO_2 concentration and water regime on stable isotope signatures, nitrogen assimilation and growth in sweet Pepper. Front. Plant Sci. 8, 2180 2018.

Shewry, P.R., 2009. Wheat. J. Exp. Bot. 60, 1537–1553.

Sissons, M., 2008. Role of durum wheat composition on the quality of pasta and bread. Food 2 (2), 75–90.

Sissons, M.J., Osborne, B.G., Hare, R.A., Sissons, S.A., Jackson, R., 2000. Application of the single-kernel characterization system to durum wheat testing and quality prediction. Cereal Chem. 77 (1), 4–10.

Soh, H.N., Sissons, M.J., Turner, M.A., 2006. Effect of starch granule size distribution and elevated amylose content on durum dough rheology and spaghetti cooking quality. Cereal Chem. 83 (5), 513–519.

Suchy, J., Lukow, O., Brown, D., DePauw, R., Fox, S., Humphreys, G., 2007. Rapid assessment of glutenin and gliadin in wheat by UV spectrophotometer. Crop Sci. 47, 91–99.

Troccoli, A., Borrelli, G., De Vita, P., Fares, C., Di Fonzo, N., 2000. Durum wheat quality: a multidisciplinary concept. J. Cereal Sci. 32, 99–113.

Van Hung, P., Yamamori, M., Morita, N., 2005. Formation of enzyme-resistant starch in bread as affected by high-amylose wheat flour substitutions. Cereal Chem. 82, 690–694.

Vicente, R., Vergara-Díaz, O., Medina, S., Chairi, F., Kefauver, S.,C., Bort, J., Serret, M.D., Aparicio, N., Araus, J.L., 2018. Durum wheat ears perform better than the flag leaves under water stress: gene expression and physiological evidence. Environ. Exp. Bot. 153, 271–285.

Wieser, H., 2007. Chemistry of gluten proteins. Food Microbiol. 24 (2), 115–119.